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Pathogenicity of *Saccharomyces cerevisiae* in Complement Factor Five-Deficient Mice

JULIE K. BYRON,[1,2] KARL V. CLEMONS,[2,3,4]* JOHN H. MCCUSKER,[5] RONALD W. DAVIS,[5] AND DAVID A. STEVENS[2,3,4]

Department of Biology, Stanford University,[1] and Division of Infectious Diseases, Department of Medicine,[4] and Department of Biochemistry,[5] Stanford University School of Medicine, Stanford, California 94305, and California Institute for Medical Research,[2] and Division of Infectious Diseases, Department of Medicine, Santa Clara Valley Medical Center,[3] San Jose, California 95128

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We have previously determined the relative virulence of isolates of *Saccharomyces cerevisiae* on the basis of differences in proliferation and resistance to clearance in CD-1 mice. These infections were not fatal. To further characterize *S. cerevisiae* pathogenesis, we studied a virulent clinical isolate, YJM128, and an avirulent nonclinical isolate, Y55, in C5-deficient mice. DBA/2N mice were infected intravenously with YJM128 or Y55, and temporal burdens of yeast cells in various organs were determined. After infection with 10^7 CFU, Y55 increased by 13-fold and YJM128 increased by 20-fold in the brain from day 0 to 3. In addition, YJM128 increased by 4-fold in the kidneys, whereas Y55 decreased by 16-fold. Both isolates declined in number in other organs. In all studies, 90% of mice infected with 10^7 CFU of YJM128 died between days 2 and 7, whereas no mice infected with equivalent numbers of Y55 died. No mice died after infection with 10^6 CFU of Y55 or YJM128. The importance of C5 was confirmed by studies using B10.D2/oSnJ ($C5^-$) mice and their congenic $C5^+$ counterparts. Again, the $C5^-$ mice were most susceptible to infection with *S. cerevisiae*, with 63% infected with YJM128 dying by day 7; no $C5^+$ mice died. No Y55-infected mice died, and mean burdens in the brain at day 14 were sevenfold lower in $C5^+$ mice than in $C5^-$ mice. Seven of 10 other *S. cerevisiae* isolates were also more virulent in DBA/2N than CD-1 mice, causing ≥40% mortality. These data indicate that C5 is a critical factor in host resistance against *S. cerevisiae* infections and further confirm the pathogenic potential of some isolates of *S. cerevisiae.*

Although commonly considered a nonpathogen, *Saccharomyces cerevisiae* has been reported recently in a number of cases of sepsis in patients with such predisposing conditions as prolonged hospitalization, immunosuppression, broad-spectrum antibiotic therapy, and foreign devices such as central venous catheters or prosthetic cardiac valves (1, 5, 8–10, 22, 23, 27). The increased number of reported cases might be a result of improved diagnosis or perhaps a willingness of the medical community to view *S. cerevisiae* as a potential pathogen. In either case, it is apparent that this yeast poses a potential problem in patients with compromised immune function.

To further define the potential pathogenicity of *S. cerevisiae*, murine models of systemic infection have been used with varying results (6, 14, 16). In earlier studies, we recovered *S. cerevisiae* from the brains of CD-1 mice in numbers as high as 10^5 CFU 14 days after infection with a clinical isolate of *S. cerevisiae*, whereas a laboratory strain was rapidly cleared; the mice infected with the latter carried greater than 1,000-fold-lower burdens of *S. cerevisiae* in the brain (6). Thus, virulence was defined as the capacity for the organism to proliferate in the brain and/or persist for an extended period of time (6). On the basis of these criteria, the degrees of virulence of various isolates of *S. cerevisiae* were compared. These results demonstrated that 8 of the 13 clinical isolates of *S. cerevisiae* tested exhibited higher degrees of virulence than did the 10 nonclinical isolates tested when inoculated into immunocompetent CD-1 mice (6). No death was observed in these animals as a result of infection due to *S. cerevisiae*. In addition, the genetics of clinical and nonclinical isolates of *S. cerevisiae* have been studied in relation to some potential virulence traits (6, 17). It was therefore of interest to examine further the role of host susceptibility in infection with *S. cerevisiae.*

Complement deficiency (in particular, of the fifth component [C5]) has been implicated in increasing susceptibility of humans and mice to fungal infections with *Aspergillus fumigatus*, *Candida albicans*, and *Cryptococcus neoformans* as well as infections caused by gram-negative bacteria (12, 13, 15, 18, 21, 24, 28). However, C5 does not appear to be important in *Histoplasma capsulatum*, *Paracoccidioides brasiliensis*, or *Coccidioides immitis* infection (3, 4, 7, 29). Purified C5 has been shown to enhance the opsonization of *S. cerevisiae* and increase the phagocytosis of it by polymorphonuclear leukocytes in vitro when added to human or mouse sera lacking this component (18, 19). In the current study, we examined the virulence of several of the previously characterized isolates in mice lacking C5 to determine whether C5 deficiency alters host susceptibility to infection with *S. cerevisiae.*

MATERIALS AND METHODS

***S. cerevisiae* isolates.** Twelve isolates of *S. cerevisiae* were used during these experiments. Eight of these were clinical isolates, namely, YJM128, YJM210, YJM273, YJM308, YJM309, YJM311, YJM436, and YJM454. Two were genetically defined laboratory strains, Y55 and YJM237, and one was a nonclinical isolate, YJM264. One isolate, YJM145, is a segregant derived from YJM128. The sources and characterization of these isolates have been described elsewhere (6, 17). Stock organisms were stored at −80°C in 40% (vol/vol) sterile glycerol in water. Prior to each experiment, stock isolates were recovered from storage and grown at ambient temperature on YPD agar slopes or broth, which contained 1%

* Corresponding author. Division of Infectious Diseases, Santa Clara Valley Medical Center, 751 S. Bascom Ave., San Jose, CA 95128. Phone: (408) 998-4557. Fax: (408) 998-2723.

yeast extract (Difco Laboratories, Detroit, Mich.), 2% Bacto Peptone (Difco), and 2% dextrose with or without 1.5% agar.

Animals. Four-week-old, C5-deficient, male DBA/2N mice (Simonsen Laboratories, Gilroy, Calif.) were the primary host used in these experiments. Additional studies on the importance of C5 in resistance to infection were done with 4-week-old male B10.D2/oSnJ ($C5^-$) and B10.D2/nSnJ ($C5^+$) mice, which are congenic for C5 deficiency (Jackson Laboratories, Bar Harbor, Maine). Mice were provided acidified water and sterilized food ad libitum.

Experimental infections. Isolates of *S. cerevisiae* were transferred from −80°C storage to YPD agar slants and grown for 48 h at room temperature. The isolates were then transferred to YPD broth and grown on a gyratory shaker at 140 rpm for 22 to 24 h at ambient temperature. Cells were harvested and washed twice with saline after centrifugation at 1,500 × *g*. The cells were counted on a hemacytometer and diluted to the desired concentration in saline. Viability was determined by serial dilution and quantitative plating onto Sabouraud dextrose agar (Difco) containing 50 mg of chloramphenicol per liter (SDAc). Plates were incubated at ambient temperature, and colonies were counted after 48 h.

To initiate infection, DBA/2N, B10.D2/oSnJ, or B10.D2/nSnJ mice were inoculated intravenously with 2×10^7, 2×10^6, or 2×10^5 viable CFU of *S. cerevisiae*. At various times postinfection, 2 to 11 surviving mice were killed by CO_2 anoxia, and the brain, spleen, liver, kidneys, and lungs were removed aseptically from each. Each organ was weighed and homogenized in 5 ml of sterile saline containing 100 IU of penicillin per ml and 100 μg of streptomycin per ml with a Tissumizer (Tekmar, Cincinnati, Ohio). Tissue homogenates were diluted serially in sterile saline and plated quantitatively onto SDAc for determination of burdens of *S. cerevisiae*. The plates were incubated for 48 h at ambient temperature, and colonies were counted. Organs for histological evaluation were placed into 10% buffered formalin and embedded in paraffin. Sections were stained with hematoxylin and eosin or Gomori methenamine silver stain.

Temporal burdens of *S. cerevisiae* in various organs were compared by the Mann-Whitney U test (25). Results from multiple experiments using the same conditions were pooled prior to evaluation. All burdens are expressed as the $\log_{10}$ geometric mean number of CFU per entire organ. Ninety-five percent confidence intervals were determined by use of the GB-STAT version 3.0 computer program (Dynamic Microsystems, Inc., Silver Spring, Md.).

In vitro cell flocculation studies. Flocculation studies of *S. cerevisiae* were done by a modification of previously described methods (26). In brief, isolates grown at room temperature on a gyratory shaker at 140 rpm for 24 h were harvested and washed twice in 5 ml of 250 mM EDTA in a centrifuge at 1,500 × *g* to disperse any preexisting aggregates (flocs) of cells. Dispersed *S. cerevisiae* cells were resuspended in 5 ml of 50 mM citrate buffer (pH 3.0) containing 5 mM EDTA, counted on a hemacytometer, and diluted to 8×10^7 cells per ml. One milliliter of 50 mM $CaCl_2$ buffer was added to 4 ml of each suspension, and the tubes were rotary inverted at 30 rpm for 5 min to induce flocculation. Flocs were allowed to settle for 5 min, and 0.5-ml samples of the upper cell suspension were taken from <5 mm below the meniscus. Samples were diluted with 2.5 ml of 250 mM EDTA, and duplicate spectrophotometer readings at A_{600} were averaged to determine the degree of flocculation of the 12 *S. cerevisiae* isolates. Those with the highest A_{600} reading were considered to have the lowest degree of flocculation.

RESULTS

***S. cerevisiae* infection in C5-deficient mice.** In a preliminary study, $C5^-$ DBA/2N mice were infected intravenously with 2×10^7 yeast cells of the virulent *S. cerevisiae* isolate YJM128 or the avirulent isolate Y55 to determine mean burdens of yeast cells in the organs. Of 11 mice infected with YJM128, 9 died between days 2 and 4 of infection (three each on days 2, 3, and 4 postinfection). The remaining two mice died on days 5 and 7, respectively. Brain, spleen, and liver samples from these mice were plated onto SDAc, and viable *S. cerevisiae* was recovered from the organs (data not shown). Other organisms were not observed after plating or upon histologic examination of tissues from infected mice, suggesting that *S. cerevisiae* infection was the cause of death.

In contrast, no Y55-infected mice died during the initial days of infection. To determine if this was a result of clearance of Y55 from the tissues, one half of the surviving Y55-infected mice were killed on days 14 and 28 after infection. The residual numbers of Y55 cells in the brain, spleen, liver, kidneys, and lungs were determined. On day 14, the numbers of recovered *S. cerevisiae* cells ranged from 0.82 to 3.34 mean $\log_{10}$ CFU per organ, with the highest numbers recovered from the brain and the lowest numbers recovered from the lungs. The kidneys, spleen, and liver had intermediate yeast cell burdens of 1.25, 1.38, and 1.83 mean $\log_{10}$ CFU, respectively. By day 28, yeast cell numbers had declined by 200-fold in the brain and by 7- to 18-fold in other organs. No viable yeast cells were recovered from the lungs of Y55-infected mice at day 28.

To confirm these results and determine whether a lower inoculum concentration could cause death, similar experiments were performed by inoculating 2×10^7, 2×10^6, or 2×10^5 CFU of YJM128 or Y55 into DBA/2N mice. Of those infected with 2×10^7 CFU of YJM128, three of five died on days 2, 3, and 5 postinfection; these results are similar to those of the initial experiment. No mice given a lower concentration of YJM128 died, and no Y55-infected mice died. On day 30, the surviving YJM128-infected mice and Y55-infected mice were killed and the viable numbers of yeast cells in the organs were determined. Yeast cell burdens recovered from mice infected with 2×10^7 CFU of YJM128 were (in mean $\log_{10}$ CFU) 3.33 in the brain, 3.81 in the spleen, 3.34 in the liver, 2.53 in the kidneys, and 2.76 in the lungs. The numbers of yeast cells recovered from 2×10^7 CFU of Y55-infected mice were similar to day 28 levels of Y55 as determined above.

S. cerevisiae burdens in mice infected with 2×10^6 CFU of either isolate are shown in Fig. 1. The numbers of yeast cells recovered from the brain on day 30 were significantly different ($P < 0.05$) and almost 650-fold higher in mice infected with YJM128 than in mice infected with Y55. Yeast cell numbers in the liver were 5-fold higher ($P < 0.05$) and those in the kidney were 27-fold higher ($P < 0.05$) in YJM128-infected mice than they were in Y55-infected mice. Numbers of yeast cells in the spleen and lung were not significantly different between the two isolates ($P > 0.05$). The numbers of yeast cells recovered from mice infected with 2×10^5 CFU of both isolates were approximately 10-fold lower than those of mice infected with 2×10^6 CFU (data not shown).

In combination with data from the previous experiment, we compiled long-term clearance profiles of YJM128 and Y55 in DBA/2N mice given an inoculum of 2×10^6 CFU (Fig. 1). Mice were infected with YJM128 or Y55, and burdens of yeast cells in the organs were determined at 4 h postinfection (day 0) as well as on days 7 and 14 (Fig. 1). On day 0, YJM128-infected mice had significantly higher numbers of yeast cells in the spleen, liver, and lung than did Y55-infected mice ($P < 0.05$); the numbers of yeast cells recovered from the kidneys were equivalent. Interestingly, Y55-infected mice had significantly higher levels of yeast cells in the brain ($P < 0.05$). The highest initial burdens of both isolates were in the liver (Fig. 1). By day 7 in the brain, YJM128 levels had increased by 13-fold and Y55 levels had increased by 3-fold. Both isolates proliferated in the brain, with YJM128 reaching significantly higher levels than Y55 by day 7 ($P < 0.05$). Although no proliferation was observed in other organs, YJM128 was present in significantly greater numbers than Y55 was on days 7 and 14 ($P < 0.05$, all organs). It is clear from these data that Y55 was cleared from the mice faster than YJM128 when this size inoculum was used; YJM128 levels remained above a mean $\log_{10}$ CFU of 3.00 on day 30 in the brain.

Early events in infection. After determining that both isolates proliferated in the brain between days 0 and 7 of infection, we further quantitated proliferation during days 0 through 3 of infection, the period during which death occurred in high-inoculum (10^7 CFU) YJM128-infected mice. The burdens of YJM128 and Y55 cells recovered from these mice are shown in Fig. 2. Proliferation of YJM128 and Y55 occurred in the brains of infected mice. Interestingly, Y55 showed increases comparable to those of YJM128 in the brain during the first 3 days of infection ($P > 0.05$). No proliferation of Y55 was observed in any other organ (Fig. 2). The slightly higher level

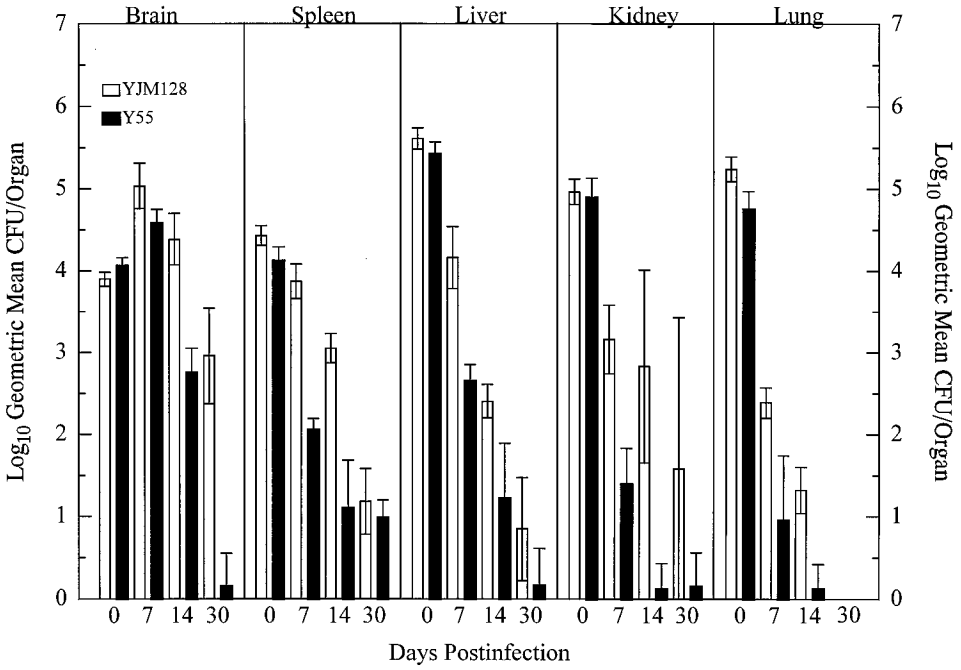

FIG. 1. Comparative burdens ($\log_{10}$ geometric mean number of CFU) in the brain, spleen, liver, kidneys, and lungs, at various times after infection with 2×10^6 CFU of YJM128 and Y55 isolates of *S. cerevisiae*. Results for YJM128 are accumulated from five mice, and results for Y55 are from five or six mice. Error bars, 95% confidence intervals.

of YJM128 in the spleen on day 1 was not significantly different from that of Y55 ($P > 0.05$). The largest difference in residual burdens between the two isolates was in the kidney, where YJM128 proliferated by fourfold and Y55 declined by fourfold from day 0 to day 3 (Fig. 2) ($P > 0.05$, day 0; $P < 0.05$, day 1 to 3). In the liver, YJM128 levels declined 6-fold from day 0 to 3, but Y55 declined more than 21-fold over the same period ($P < 0.05$, day 0 to 3). The increase of YJM128 in the lungs on day 3 was from the results of a single mouse, because of the death of the other mice in this group on day 2,

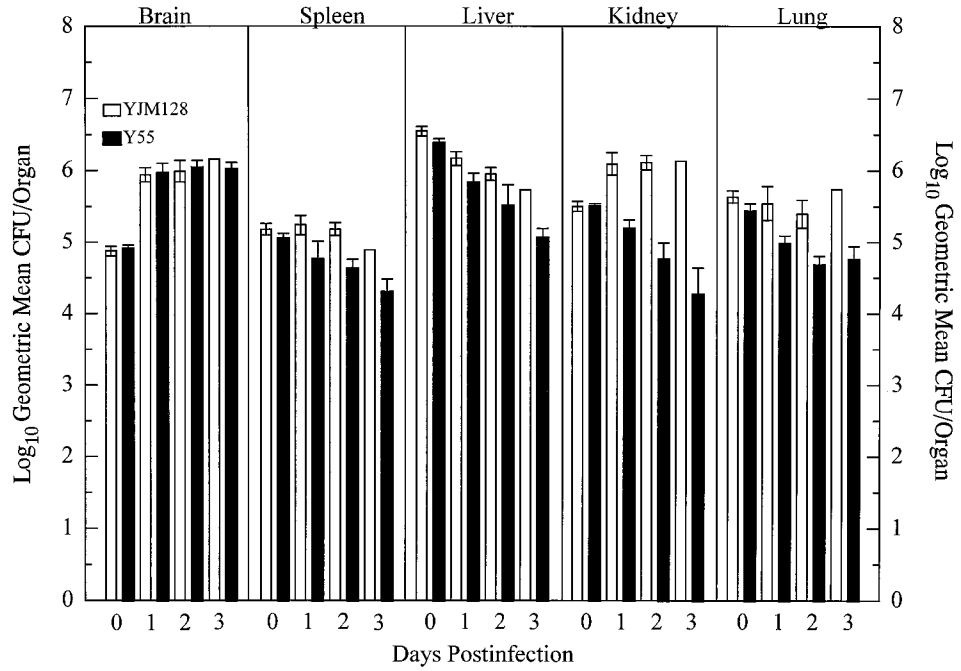

FIG. 2. Comparative burdens ($\log_{10}$ geometric mean number of CFU) in the brain, spleen, liver, kidneys, and lungs at various times after infection with 2×10^7 CFU YJM128 or Y55 isolates of *S. cerevisiae*. Results for YJM128 and Y55 on day 0 are from three experiments (15 mice). Day 1 and 2 results for both isolates are from two experiments (10 mice), and day 3 results are from one (YJM128) and five (Y55) mice. Error bars, 95% confidence intervals.

TABLE 1. Comparative burdens (log_{10} geometric mean number of CFU) in the brains of mice congenic for C5 on day 14 after infection with 2×10^7 CFU of isolate YJM128 or Y55 of *S. cerevisiae*

Isolate	Mouse strain	No. of mice surviving/ total no.	Log_{10} geometric mean CFU/organ (L_1–L_2, 95% CI)[a]				
			Brain	Spleen	Liver	Kidney	Lung
YJM128	B10.D2/oSnJ ($C5^-$)	3/8	5.56 (4.66–6.46)	4.22 (3.89–4.54)	3.35 (2.24–4.47)	4.18 (4.00–4.35)	2.75 (1.49–4.01)
	B10.D2/nSnJ ($C5^+$)	6/6	5.40 (5.26–5.53)	3.50 (3.37–3.63)	2.64 (2.38–2.90)	2.63 (2.12–3.14)	1.52 (1.30–1.73)
Y55	B10.D2/oSnJ ($C5^-$)	5/5	3.32 (2.77–3.87)	1.98 (1.76–2.21)	1.97 (1.67–2.28)	1.15 (0.29–2.02)	0.14 (0.00–0.54)
	B10.D2/nSnJ ($C5^+$)	5/5	2.50 (1.85–3.15)	1.61 (1.34–1.87)	1.94 (1.80–2.09)	0.30 (0.00–0.80)	0.14 (0.00–0.54)

[a] CI, confidence interval. L_1 and L_2, lower and upper limits, respectively, of the 95% confidence interval.

and thus may be an inaccurate representation of the yeast cell levels. The slight increase in Y55 in the lungs on day 3 postinfection was not significantly higher ($P > 0.05$) than day 2 levels of Y55.

Experiments with congenic mice. To determine if C5 deficiency was a key factor in the results described above, experiments using B10.D2/oSnJ ($C5^-$) and B10.D2/nSnJ ($C5^+$) mice, which are congenic for C5, were done. After inoculation with 2×10^7 CFU of YJM128 or Y55, infection-related deaths occurred only in the B10.D2/oSnJ group of mice infected with YJM128 (Table 1). On day 14, all surviving mice were killed and the mean levels of yeast cells in the organs were determined. In mice infected with YJM128, all burdens of yeast cells in the organs of surviving B10.D2/oSnJ mice were significantly higher than those recovered from B10.D2/nSnJ mice on day 14 ($P < 0.05$, all organs) (Table 1). In mice infected with Y55, burdens of yeast cells recovered from the brain, spleen, and kidneys of B10.D2/oSnJ ($C5^-$) mice were significantly higher than those recovered from B10.D2/nSnJ ($C5^+$) mice ($P < 0.05$) (Table 1). Burdens recovered from the liver and lung were equivalent in mice infected with Y55 ($P > 0.05$).

Histology. In addition to determination of recoverable yeast cells in the organs, histological studies were performed on the brains and kidneys of mice on days 0, 1, and 2 after infection with YJM128 or Y55. Examination of hematoxylin-and-eosin- and Gomori methenamine silver-stained sections of these organs gave an overall impression of proliferation of both YJM128 and Y55 in the brain. On day 0, the yeast cells were seen in the capillaries of the brains of animals infected with either YJM128 or Y55 (Fig. 3a and b). No pseudohyphal formation was observed. On day 1, almost no cellular response was seen in the brains of Y55-infected mice (Fig. 3c). However, in the brains of YJM128-infected mice, small clusters of yeast cells were seen in association with one or two microglial cells, although no proliferation of glial cells was evident (Fig. 3d). By day 2, in the brains of Y55-infected mice, there was still little to no cellular response to the organisms observed (Fig. 3e). In contrast, small focal areas of microglial cell proliferation were evident in the brains of YJM128-infected mice; however, no widespread response was seen (Fig. 3f). No cellular response was observed in the kidneys of animals infected with either organism (data not shown). Upon general inspection, YJM128 appeared more invasive into parenchymal tissue of the brain than Y55, which was localized primarily to the capillaries (data not shown).

Screening of isolates for virulence. Having determined that C5 is an important factor in host resistance to infection with YJM128 and Y55, other clinical and nonclinical isolates of *S. cerevisiae* were also tested in DBA/2N mice. This was done to determine whether the virulence of other isolates was also higher in a C5-deficient host. DBA/2N mice were infected with 2×10^7 CFU of each of the isolates listed in Table 2. One hundred percent of mice infected with two of these isolates, YJM311 and YJM210, died by day 1 postinfection. Two other isolates, YJM309 and YJM454, caused 100% of the infected mice to die by days 4 and 5 of infection, respectively (Fig. 4). Three of the remaining isolates, YJM264, YJM308, and YJM145, also caused the death of infected DBA/2N mice. However, 30, 40, and 60% of infected animals, respectively, lived until day 14, when all surviving mice were killed and residual yeast cell numbers in the organs were determined (Fig. 4; Table 2). Of the three isolates that did not cause death, YJM237 was determined to have the lowest mean log_{10} burdens of yeast cells in the organs; these levels were statistically lower than those in the next-most-virulent isolate, Y55 ($P < 0.05$). Of the nonlethal isolates, YJM273 had the highest mean burdens in the organs. However, only in the kidney and lung were yeast cell burdens significantly higher than those in YJM436-infected mice ($P < 0.05$) (Table 2). Overall, the rank order of virulence for all isolates of *S. cerevisiae* tested in DBA/2N mice was YJM311 ≥ YJM210 > YJM309 > YJM454 > YJM128 > YJM264 > YJM308 > YJM145 >> YJM273 > YJM436 > Y55 >> YJM237.

In vitro flocculation. During our studies, we observed that YJM128 was flocculent, whereas Y55 was not, which raised the possibility that the flocculent isolate caused death by physical obstruction of capillary beds and, thus, had an influence on the virulence of *S. cerevisiae* isolates in $C5^-$ animals. No correlation was found between this phenotype and virulence in DBA/2N mice (data not shown). The rank order of flocculence was found to be YJM145 > YJM311 > YJM128 > YJM436 > YJM237 > Y55 > YJM273 > YJM309 > YJM454 > YJM308 > YJM264 > YJM210.

DISCUSSION

The current studies were done as part of a larger project of defining the genetic traits responsible for virulence of *S. cerevisiae*. In previous work, we demonstrated that isolates of *S. cerevisiae* represented a continuum of comparative virulence rather than a clear virulent or avirulent pattern (6). However, relative virulence was based solely on temporal profiles of proliferation and clearance of yeast cells from the tissues of normal mice, since no infection-related deaths were caused by any isolate of *S. cerevisiae* tested (6). Although these results indicated that there were clear differences in virulence among isolates, the lack of infection-induced deaths was suggestive of a high innate resistance of normal mice to this organism. One factor that contributes significantly to innate resistance is complement.

The components of the complement cascade have a number of effector functions in the immune response, including induction of chemotaxis, opsonization of pathogens, and formation of a membrane attack complex (11). The importance of C5 in resistance to fungal infections has been demonstrated in vivo (12, 13, 15, 20, 24). In vitro, C5 has been shown to be important

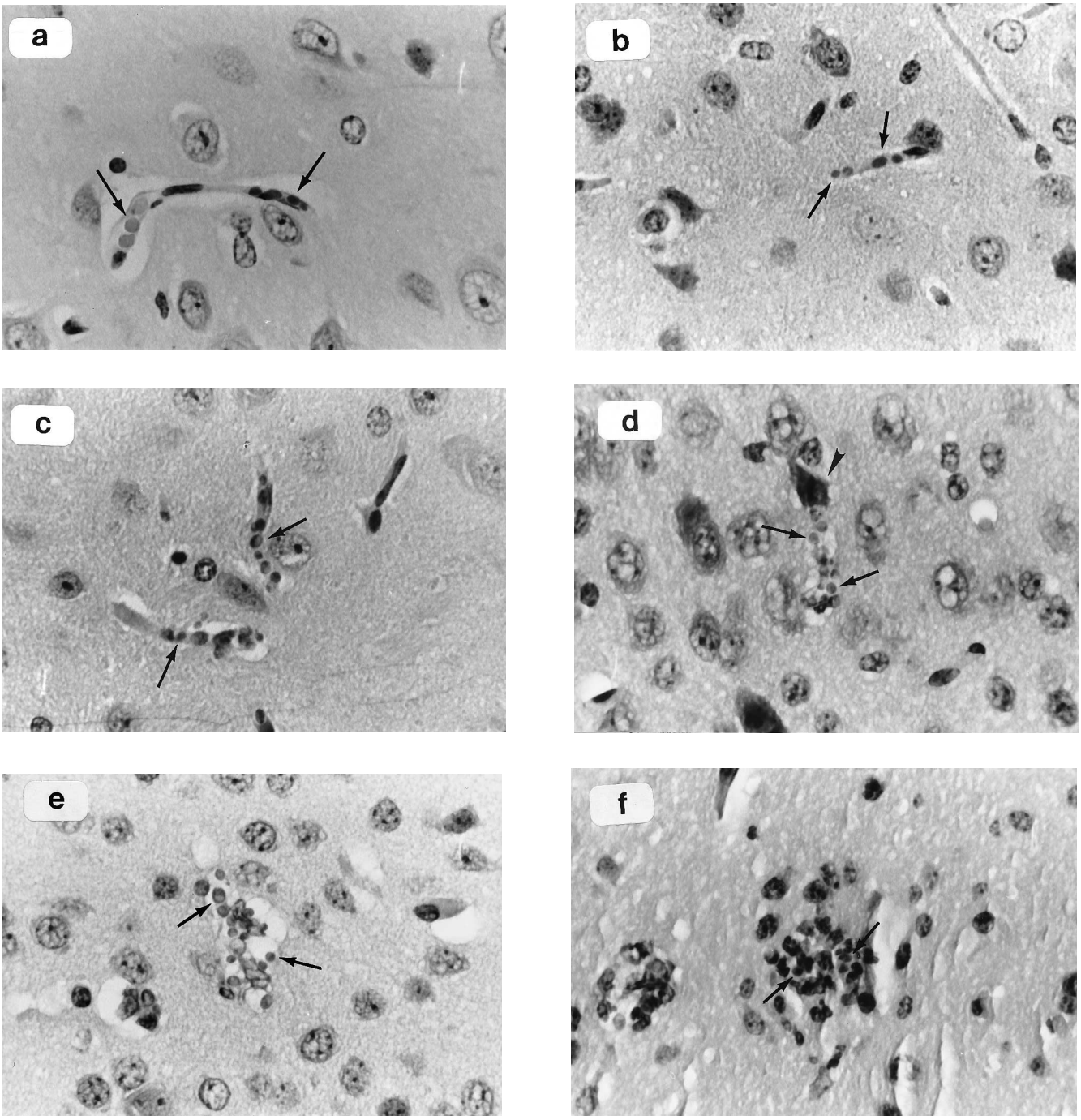

FIG. 3. Histologic studies of DBA/2N mouse brain at days 0, 1, and 2 after infection with 2×10^7 CFU of Y55 or YJM128. (a and b) Y55 (a) and YJM128 (b) yeast cells in the capillaries of the brain on day 0 (arrows); (c) Y55 yeast cells in the brain on day 1 (arrows); (d) YJM128 yeast cells in the brain on day 1 (arrows) (note microglial cell [arrowhead]); (e) Y55 yeast cells in the brain on day 2 (arrows); (f) YJM128 yeast cells in the brain on day 2 (arrows) (note focal point of inflammation). Brains were stained with hematoxylin and eosin (see Materials and Methods). Magnification, ca. ×320.

in the opsonization of various fungi (2, 18, 19, 21). In particular, C5 and not C3 has been demonstrated to be essential for opsonization and subsequent phagocytosis of viable *S. cerevisiae* (19); trypsinized baker's yeast or zymosan particles were found not to require C5 for opsonization (19). Thus, we examined the role of complement and, in particular, C5 in innate resistance to infection with *S. cerevisiae* with the thought that C5 deficiency might lead to decreased phagocytosis in vivo, which would allow for greater proliferation of *S. cerevisiae* than had occurred in normal animals (6).

Our results clearly demonstrate that C5 is important in the clearance of infection with some strains of *S. cerevisiae* by a murine host. The capacity of the virulent isolate YJM128 to cause the death of $C5^-$ DBA/2N or B10.D2/oSnJ mice, but not of congenic $C5^+$ B10.D2/nSnJ mice, clearly shows that a functional complement cascade is a critical factor in host resistance

TABLE 2. Comparative burdens ($\log_{10}$ geometric mean number of CFU) in the brains of DBA/2N mice on day 14 after infection with 2×10^7 CFU of various isolates of *S. cerevisiae*

Isolate	No. of mice surviving/ total no.	$\log_{10}$ geometric mean CFU/organ (L_1–L_2, 95% CI)[a]				
		Brain	Spleen	Liver	Kidney	Lung
YJM436	10/10	4.89 (4.65–5.11)	3.09 (3.88–4.10)	3.50 (3.36–3.65)	3.89 (3.57–4.02)	3.08 (2.93–3.23)
YJM145	6/10	4.15 (2.71–5.59)	3.15 (1.52–4.78)	2.39 (1.13–3.65)	2.23 (1.74–2.72)	1.79 (0.85–2.73)
YJM237	11/11	1.45 (0.79–2.11)	0.58 (0.20–0.97)	0.64 (0.16–1.12)	0.33 (0.00–0.74)	0.74 (0.19–1.29)
YJM273	5/5	5.13 (4.93–5.34)	3.99 (3.79–4.18)	3.63 (3.48–3.78)	3.09 (3.00–3.18)	2.88 (2.59–3.16)
YJM308	3/5	2.96 (0.00–23.06)	2.34 (0.00–10.98)	1.80 (0.00–10.50)	2.01 (0.00–27.48)	1.06 (0.00–14.46)
YJM264	2/6	2.15 (0.00–9.32)	1.40 (0.00–4.07)	1.65 (1.58–1.71)	1.82 (0.00–7.98)	1.32 (1.32–1.32)
YJM311	0/10	NA[b]	NA	NA	NA	NA
YJM309	0/5	NA	NA	NA	NA	NA
YJM454	0/6	NA	NA	NA	NA	NA
YJM210	0/5	NA	NA	NA	NA	NA

[a] CI, confidence interval. L_1 and L_2, lower and upper limits, respectively, of the 95% confidence interval.
[b] NA, not available.

to *S. cerevisiae*. The requirement for functional C5 seems to be essential especially during the first week of infection, the time period during which deaths occurred as a result of infection with YJM128 as well as seven of the other isolates tested in DBA/2N mice. However, not all of the isolates of *S. cerevisiae* tested caused mortality. Because these isolates were cleared from the tissues, as in previous studies done with CD-1 mice (6), C5 does not appear to be an absolute requirement for innate resistance against all isolates of *S. cerevisiae*. These results are similar to those confirming the requisite role of functional C5 in innate resistance to *C. neoformans* (24) and *C. albicans* (12, 15); C5 has been shown to be less important in the development of acquired resistance to *C. albicans* (12, 15). Our results with *S. cerevisiae* are in contrast to studies with other fungi where the genetic background of the host rather than complement was indicated to be more important in resistance to infection (3, 4, 7, 29). It should be noted that we examined multiple isolates of *S. cerevisiae* and found that not all of them increased in virulence in C5-deficient DBA/2N mice. Because studies done with other fungi (3, 4, 7, 12, 15, 24, 29) have relied primarily upon the results from a single isolate, it would be of interest to test additional isolates of each organism to see if the reported patterns of virulence and host susceptibility remain constant.

The key role of complement rather than haplotype in resistance to infection by *S. cerevisiae* was also found by infecting BALB/c mice, which are *H-2*^{d} like DBA/2N, B10.D2/oSnJ, and B10.D2/nSnJ mice. *S. cerevisiae* did not cause lethal infection of BALB/c mice, which are complement-sufficient animals (data not shown). Thus, three strains of mice with the same haplotype differed in resistance to *S. cerevisiae* primarily if not solely as a result of the presence or absence of C5. The data obtained from the DBA/2N studies on temporal clearance of *S. cerevisiae* further support this when compared with previously published data regarding CD-1 immunocompetent mice (6). Infections with 2×10^7 CFU of YJM128 caused the death of DBA/2N mice but not of CD-1 mice. Furthermore, infections with 2×10^7 CFU of Y55 (the avirulent isolate) led to 20-fold-

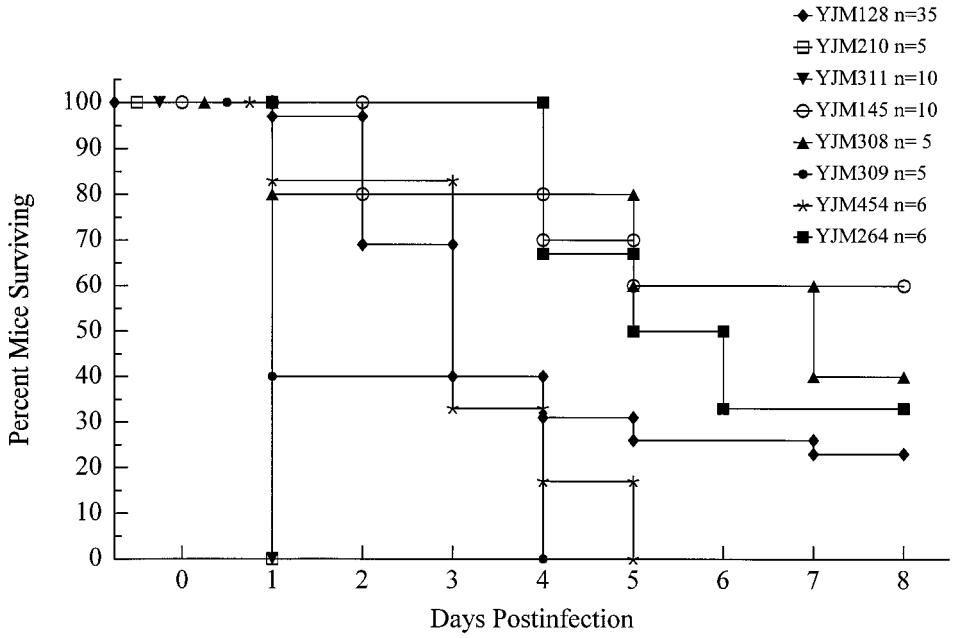

FIG. 4. Mortality in DBA/2N mice after infection with 2×10^7 CFU of various isolates of *S. cerevisiae* causing death. Four isolates did not cause death in DBA/2N mice with this size inoculum.

higher burdens of yeast cells in the brains of DBA/2N mice on day 14 than in CD-1 mice (6). Even on day 28, DBA/2N mice retained twofold-higher numbers of Y55 in the brain than did CD-1 mice (6). Thus, these data also support our finding that C5 has a key role in the host response to infection with *S. cerevisiae*.

There appear to be four degrees of virulence when the 12 isolates tested are placed in categories according to the capacity to produce death and/or persist in the brain to day 14 in DBA/2N mice. The first may be described as high virulence and was characterized by the death of more than 80% of the infected mice; YJM311, YJM210, YJM309, YJM454, and YJM128 fall into this category. The second group contains those isolates classified as having intermediate virulence. These isolates, YJM264, YJM308, and YJM145, caused the death of less than 80% of DBA/2N mice. The third group, containing isolates YJM273, YJM436, and Y55, is one of low virulence. These isolates proliferated to high numbers in the brain; however, no deaths resulted from infection. The fourth group is considered avirulent. YJM237 fell into this category because of the low number of yeast cells recovered from the organs at day 14. Comparison of this grouping with the previously assigned virulence groupings of the same isolates in CD-1 mice (6) shows that some isolates did not display increased virulence. YJM436 is one such isolate. When the mean $\log_{10}$ CFU of *S. cerevisiae* cells recovered in each mouse strain at day 14 are compared, they are very similar, i.e., 4.8 in CD-1 mice (6) and 4.89 in DBA/2N mice. A similar situation was found with YJM273. Of the 12 isolates tested, 10 were more virulent in DBA/2N mice than they were in CD-1 mice (6). Comparison of the relative rank orders of virulence for DBA/2N and CD-1 mice of the other isolates showed that YJM436 changed from one of high virulence for CD-1 mice (6) to one of low virulence for DBA/2N mice. Conversely, other isolates (e.g., YJM210) switched in rank from comparatively low virulence to high virulence.

The observation that all isolates did not display the same rank order of virulence in DBA/2N mice as that previously demonstrated in CD-1 mice (6) may result from a difference in the traits that make a particular isolate virulent. Some isolates, which tend to be opsonized and eliminated via complement activation in normal hosts, may be able to proliferate to a greater degree in the absence of C5. Others, which have means for avoiding clearance through complement-related mechanisms, may not be affected by the absence of a complete complement system.

We have shown previously that some polygenic traits are associated with virulence (6, 17). During the course of our current studies, we observed that some isolates displayed the common phenotypic trait of flocculence. Although our assays were not done under strict physiological conditions, flocculence is a lectin-binding event requiring calcium ions and has been demonstrated to occur from pH 2 through 9 in various buffer systems (26). Thus, we chose to use a previously described assay rather than develop a novel system utilizing a different buffer. Our ranking of isolates by degree of flocculation shown in vitro did not, however, correlate with the rank order of virulence. Thus, the capacity of an isolate to cause death does not seem to be simply a result of physical occlusion (with possible induction of a stroke event) of the capillaries. Furthermore, the histological data suggested that the DBA/2N mice did not mount the same level of inflammatory response to a virulent isolate (YJM128) as they did to an avirulent isolate like Y55 that does not cause death. Perhaps paradoxically, the response to YJM128 was observed to be more intense and the organism was observed to be more invasive into the parenchyma of the brain, with a corresponding proliferation of microglial cells. In contrast, the less invasive isolate Y55 did not appear to induce microglial cell proliferation and is probably cleared by blood monocytes or polymorphonuclear leukocytes. These observations further support the idea that isolates of *S. cerevisiae* differ in their capacity to cause disease.

The DBA/2N model presented here may be developed into a method of characterizing and differentiating *S. cerevisiae* isolates with respect to virulence since the capacity to proliferate in a C5-deficient host appears to be isolate specific. Since a possible end point is death, less time and technical effort may be necessary to complete the screen. Because the genetics of *S. cerevisiae* are well defined and amenable to experimental manipulation, fungal virulence factors may be easily studied in this system.

In conclusion, we have shown that C5 is important in the early innate host response to infection, with some isolates of *S. cerevisiae* able to cause mortality. However, in comparing the relative degrees of virulence of the various isolates in C5-sufficient and C5-deficient mice, it is apparent that different isolates of *S. cerevisiae* have developed differing genetic strategies for the manifestation of virulence, regardless of the status of the host response. *S. cerevisiae* has been added to the list of emerging pathogens to which an immunocompromised host is susceptible and must now be looked at in the same light as other more common fungal pathogens.

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